Modified Procedure for Isolation of a Major Swine Whey Protein

Abstract

A salt fractionation procedure for the isolation of a polymorphic whey protein in sow's milk was modified to include anion exchange chromatography. The protein, homologous to ruminant β-lactoglobulin, was crystallized from pooled milk and its identity to the previously isolated polymorphic whey protein was verified by alkaline and acid gel electrophoresis.

Kraeling and Gerrits (5, 6) have described a polymorphic protein in sow's whey. These workers have reported the distribution in three swine breeds, one of which was a crossbreed, of the genetic variants AA and BB according to their mobilities in alkaline gel electrophoresis. Kalan et al. (1, 2, 3) isolated each of the polymorphs from milk from sows found to be homozygous for the individual polymorphs. They have partially characterized these proteins for amino acid composition, C-terminal and N-terminal amino acids, molecular weight and isoionic point. The authors made a case for homology of the proteins with ruminant β-lactoglobulin. More recently, Kessler and Brew (4) reported on the isolation and partial characterization of a swine whey protein similar to the AA variant reported by Kalan et al. (1, 2, 3). They also have concluded homology with ruminant β-lactoglobulin for the protein they described. Kessler and Brew (4) isolated their protein by column chromatography with G-100 Sephadex in 50 mM NH_4HCO_3, pH 8.8, and DEAE-cellulose with a linear NaCl gradient in Tris-HCl buffer, pH 7.8.

The original isolation by Kalan et al. (3) of the polymorphic swine whey proteins was based on salt fractionation and, finally, crystallization from 55% saturated $(NH_4)_2SO_4$ solutions. The procedure for the isolation of these proteins has been modified to include column chromatography and our report is this modification.

Experimental Procedures

Two gallons of pooled, raw swine milk were used as the starting material, and a salt fractionation was carried out through precipitation with 2.3 M $(NH_4)_2SO_4$, that is, through Step 7 of the method of Kalan et al. (3). This precipitate, obtained by addition of solid $(NH_4)_2SO_4$, was lyophilized, dialyzed and 1 g applied to a DEAE-cellulose microgranular anion exchange resin. The resin after recycling with 0.5 N HCl and 0.5 N NaOH and thorough rinsing, was washed with the starting buffer 0.005 M potassium phosphate, pH 8.3. The resin was poured into a 2 by 50-cm column and equilibrated overnight by pumping the starting buffer through the column with a positive displacement pump. The column was operated at 8 C and flowed 52.5 ml per hour, collecting 17.5 ml per tube. The effluent was monitored by recording the absorbance at 280 nm. Stepwise elution was employed with potassium phosphate buffers at constant pH 8.3 and concentrations of 0.005, 0.04, 0.07, 0.10, 0.15, 0.20 and 0.50 M.

Results and Discussion

Initial experiments indicated that all the protein was eluted before the addition of 0.2 M phosphate. Therefore, subsequent columns were operated with only the first five phosphate buffers. Figure 1 is a chromatograph showing most of the protein, approximately 70%, eluting with 0.07 M phosphate. Fraction 4, consisting of at least three species distinguishable by gel electrophoresis, contains the protein of interest, shown in Figure 2 on an alkaline gel electrophoretic pattern in Slot 4. The patterns shown in Slots 1 and 2 probably represent other proteins of swine milk, originating either in the whey or in the casein. Likewise, the pattern shown in Slot 3 also represents other swine milk proteins with a small amount of the protein of interest. Although this is known to be a polymorphic protein from previous work (1, 2, 3), no effort was made to distinguish the A and B proteins in the present study. Fractions 5 and 6 of Figure 1 contained insufficient quantities of protein for further characterization. The method of electrophoresis was essentially that of Peterson (7) with 8% polyacrylamide gels with Tris-4.5 M urea, pH 9.2. The major portion of the proteins which eluted with 0.07 M phosphate (Fraction 4), amounting to 497 mg and containing the swine whey protein reported by Kalan et al. (1, 2, 3), was rechromatographed on the same type column as above, using now 0.04, 0.055, 0.07, 0.15 and 0.3 M phosphate buffers, stepwise. Figure 3 shows the results of this procedure and the gel pattern is in

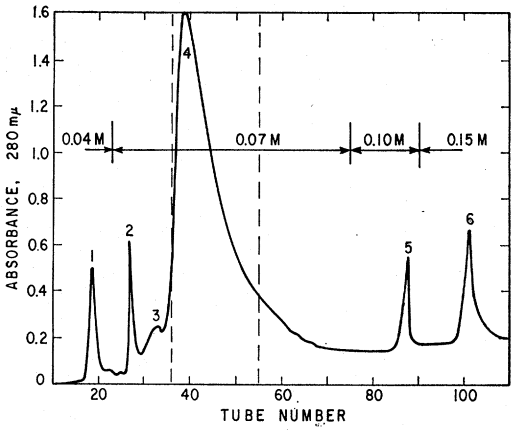

FIG. 1. Elution diagram of swine whey protein on DEAE-cellulose, stepwise elution in potassium phosphate buffer, pH 8.3. See text for conditions of elution.

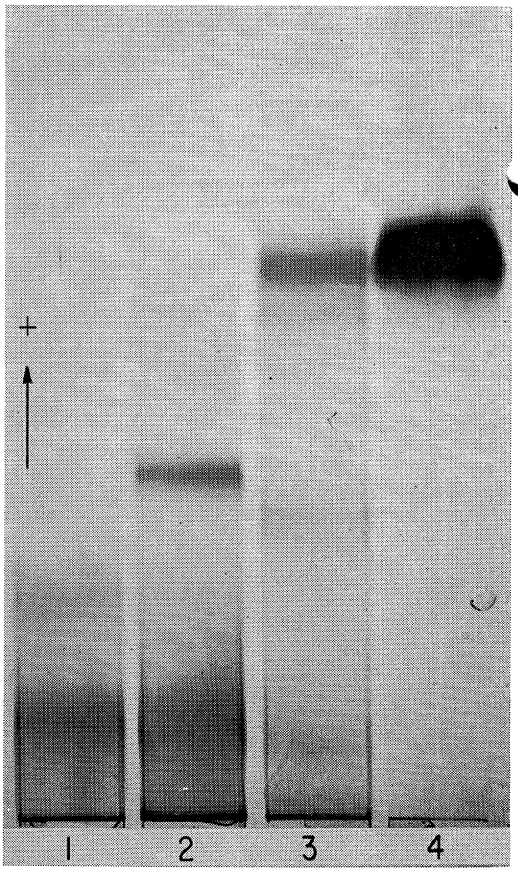

FIG. 2. Alkaline gel electrophoresis pattern showing the separation indicated in Fig. 1. See text for conditions of electrophoresis.

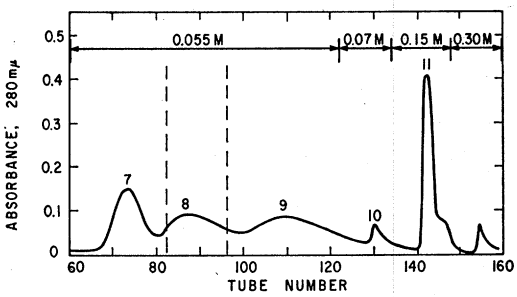

FIG. 3. Fraction 4, Fig. 1, rechromatographed under the same conditions except for a variation in the elution program. See text for variation in elution program.

Figure 4a. The yields were: Fraction 7, 33 mg; Fraction 8, 80 mg; Fraction 9, 96 mg; and Fraction 10, 25 mg. Fractions 8, 9 and 10 yield similar, if not identical, electrophoretic patterns (Fig. 4a), suggesting aggregation of the protein on DEAE-cellulose chromatography. Passage through Sephadex G-75 and G-100 with water elution failed to remove traces of a fast-moving component from Fraction 9 and a slow-moving component from Fraction 8, which component is not apparent in Figure 4a. The appearance of more than one peak eluted from Sephadex, each displaying the same gel pattern, suggested aggregation of the protein under these conditions.

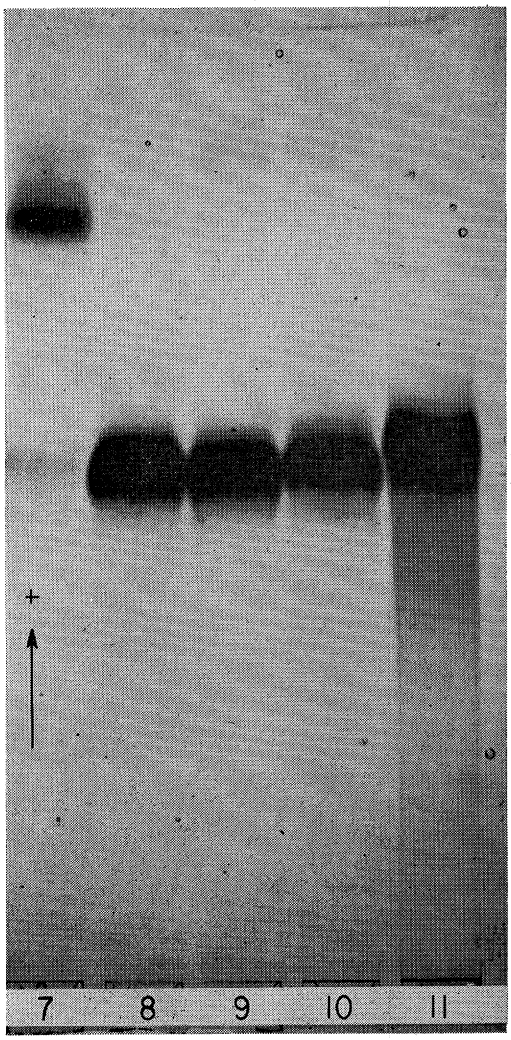

FIG. 4a. Alkaline gel electrophoresis pattern of the separation indicated in Fig. 3. Conditions of electrophoresis as in Fig. 2. Fraction 8 was crystallized as described in text.

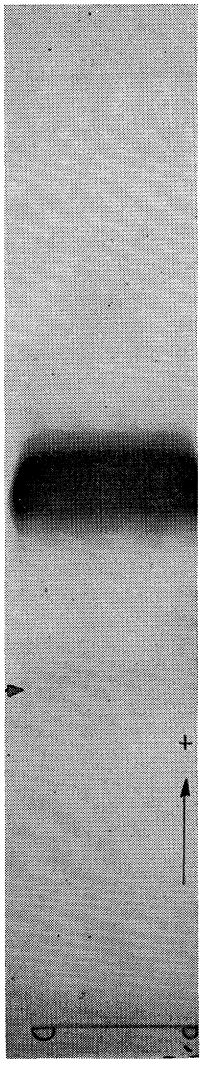

FIG. 4b. Gel pattern of the crystalline swine whey protein obtained from Fraction 8. Conditions of electrophoresis as in Fig. 2. The arrow indicates a faint slow-moving band.

The swine protein obtained by DEAE-cellulose chromatography (Fraction 8) could be crystallized from $(NH_4)_2SO_4$ solutions, as described by Kalan et al. (3), in the same crystal habit of small needles. The gel pattern of the crystalline material is shown in Figure 4b. Inspection of this gel pattern reveals the faint slow-moving band which persisted through crystallization. The protein isolated by column chromatography was identical by acid and alkaline gel electrophoresis to the swine polymorphs isolated previously. This finding would also support the work of Kessler and Brew (4) whose isolation of the same protein was completed by chromatography.

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